ALVEOLAR DAMAGE: EPITHELIAL DAMAGE AND ENDOTHELIAL DAMAGE

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Abstract: The alveolar damage produced by a variety of toxic agents, whether air-borne or blood-borne, progresses to a sequence of nonspecific changes histologically regarded as diffuse alveolar damage. Ultrastructurally, their alveolar damage can be divided into epithelial and endothelial type according to their target cells. The epithelial and endothelial type of alveolar damage was demonstrated in the experimental models of paraquat and monocrotaline toxicity, respectively. The two types of alveolar damage show different courses in the development of the sequent changes, which may influence the time courses and the patterns of final alveolar fibrosis. However, the endothelial type of alveolar damage may also induce secondary injury to the epithelial cells, so that the damage of this type finally becomes similar to that in the epithelial type. The mechanism of secondary epithelial damage was discussed on the basis of the antioxidation function of the lung. In many pneumotoxins, however, the detailed pathogenesis of alveolar changes remains to be clarified. (J Toxicol Pathol 2: 223-240, 1989)

Key words: Diffuse alveolar damage, Lung injury, Paraquat, Monocrotaline, Ultrastructure

Introduction

Diffuse alveolar damage (DAD) is a common histological feature of acute toxic injury to the lung, and can develop to lung fibrosis in the late stage. The lung injury can be induced by a variety of pneumotoxic agents, which include toxic inhalants, drugs and toxic ingestants, and radiation. The pathologic manifestations of lung injury have been generally believed to be similar for any one of these agents, whether they are blood- or air-borne, so that pathologically it has been called DAD as a descriptive term. In humans, DAD is also the usual underlying pathology of adult respiratory distress syndrome (ARDS) which is caused by a variety of indirect agents, such as shock, near drowning, sepsis, uremia, and heat, although in most cases the etiology is not clearly understood.

The histologic appearance of DAD can be divided into two stages, exudative and proliferative, although they usually overlap. The exudative stage is the early histologic change and is characterized by edema, exudation, and formation of hyaline membrane. The fulminant feature in this stage is diffuse alveolitis associated with hyaline membrane. In the proliferative stage fibroblasts proliferate in the alveolar regions and finally lung fibrosis develops as a result of the repair process. However, it should be noted that the changes of DAD are not necessarily progressive from the exudative to the organizing stage, because a mild injury may cease and normal lung structure can be completely recovered in the early stage.

However, ultrastructural studies of reactions to the various pneumotoxic agents have suggested that each agent initially affects different cell types in the alveolar walls according to their susceptibility to injury. Some agents initially affect either the epithelial or endothelial cells of the alveolar walls, while others can injure both types of cell from the initial stage. From this point of view,
pneumotoxic agents can be divided into three groups: epithelial, endothelial, and both. It is very interesting to observe the development from the initial damage of different types of cell to the common histologic changes that are called DAD. It is enough to note that the alveolar damages evolve in a different manner or course depending on the target cell types but that the common responses occur finally against any agent, whether the initial target cell is endothelial or epithelial. Therefore, it is also important to know what changes are essential for the development of the histologic features called DAD.

These findings were obtained only from ultrastructural studies based on experiments causing lung injuries in animal models. Paraquat and monocrotaline are the best available examples of agents causing initially epithelial and endothelial damage, respectively. In this paper, therefore, we will describe our ultrastructural observations on the changes induced by paraquat and monocrotaline and then discuss how the secondary or sequential changes develop, focusing on the difference in the initial damage.

Normal structure of alveolar walls

The alveolar walls consist of a network of capillaries, their supporting connective tissue, and a lining epithelium (Fig. 1). The alveolar capillaries are arranged alternately on the cross sections and are in close contact with the epithelium in the part, which forms the blood-air barrier (morphologically the thin part of the alveolar septum). The intervening connective tissue space is absent along this thin part of the alveolar septum. Its absence significantly reduces the width of the diffusion barrier to oxygen and carbon dioxide. The opposite sites to the capillaries are supported by thin connective tissue containing a few collagen fiber and septal cells (the thick part of the alveolar septum). The septal cells are not only indicative of their character as lipid-storage cells and myofibroblasts because of the presence of lipid droplets\(^1\) and myofilaments\(^2\) in the cytoplasm, but also are the precursor cells of fibroblasts which may proliferate in alveolar fibrosis. The epith-

Fig. 1. Electron micrograph of normal rat lung. Capillaries (C) reside alternately on the sides of the alveolar wall under the lining epithelial layer and form on the surface sides the thin parts (open star) which consists of thin double membranes of endothelial and epithelial cells and a fused single basal lamina. Other parts, the thick parts (closed star), contain connective tissue fibers and extending septal cells. The septal cells (S) contain lipid droplets (arrowhead) and myofilaments. ×5,225.
Epithelium consists of thin type 1 pneumocytes and cuboidal type 2 pneumocytes. The latter epithelial cells contain lamellar bodies which are the source of surfactant. Because of the lack of mitotic ability in the type 1 pneumocytes, the repaired epithelium following epithelial loss is believed to be derived from the type 2 pneumocytes.

Epithelial damage to the alveoli

Some pneumotoxic agents, such as paraquat, trisodium citrate, N-methyl-N-nitrosothiourea, ammonium sulfate, and cyclophosphamide, have a severe effect on the epithelial rather than the endothelial cells of the alveolar wall from the beginning. Paraquat (1, 1'-dimethyl 4, 4'-bipyridylium dichloride), a herbicide, is believed to be one of the typical toxins which selectively injure the alveolar epithelial cells. It is generally agreed that there is no ultrastructural evidence of primary changes caused by paraquat in pulmonary capillary endothelial cells. It has profound and usually lethal pulmonary toxicity in man after suicide attempts or accidental ingestion. The lung injury is reproducible in laboratory animals after feeding or injection of paraquat. Regarding the mechanism of paraquat toxicity, it is generally agreed that the compound undergoes a cyclic reduction/oxidation leading to the activation of molecular oxygen and the consumption of cellular-reducing equivalents in the alveolar epithelial cells. In this article we will show as a model of the epithelial damage the sequential changes in the lungs of rats treated with a single i.p. injection of paraquat (40 mg/kg).

On the histologic level, the paraquat-injured lung shows the typical features of DAD. The exudative changes of the alveoli are at their peak on the 5th day after the injection, and the proliferative or organizing changes begin on the 7th day. In the exudative stage, intra-alveolar edema is followed by formation of hyaline membrane, which is characteristic of DAD, and having macrophages migrate into the alveolar spaces (Fig. 2a). Later, fibroblastic cells gradually increase in number in the alveolar spaces as macrophage infiltration ceases. Finally a remodeling of alveolar structure occurs as a result of “intra-alveolar” fibrosis and collapse of the original alveolar lumen (Fig. 2b). The alveolar ducts, on the other hand,
Fig. 3. Rat paraquat lung. Hydropic swelling of nucleus and cytoplasm of the alveolar epithelial cell (Ep) is the initial change which is found 6 hours after i.p. injection of paraquat. The endothelial cells of capillaries (C) are structurally normal. AL: alveolar lumen. ×6,650.

Fig. 4. Rat paraquat lung (2 days after injection). In the lower part of the figure (open star) epithelial cells are severely degenerated and in the upper part (arrow-heads) the alveolar surface is completely denuded. The endothelial cells (En) of capillaries (C) show normal features. ×5,700.
Fig. 5. Rat paraquat lung (3 days after injection). In this figure the alveolar surface is denuded throughout. AL: alveolar lumen, C: capillaries. ×7,125.

Fig. 6. Rat paraquat lung (3 days after injection). Hyaline membrane (Hy) is present on the denuded alveolar surface. AL: alveolar lumen, C: capillary. ×6,650.
Fig. 7. Rat paraquat lung (3 days after injection). High magnification of hyaline membrane (Hy) shows a mass of cell debris, which is probably derived from the injured epithelial cells. The epithelial basal lamina is exposed beneath the hyaline membrane (arrowheads). ×10,925.

Fig. 8. Rat paraquat lung (5 days after injection). Repaired epithelial cells (R) which originate from type 2 pneumocytes are spreading along the remaining basal laminae. Open star: mitotic figure of pneumocytes. AL: alveolar lumen. ×1,425.
Fig. 9. Rat paraquat lung (5 days after injection). This shows an area of severe cell infiltration of the peripheral lung. Macrophages (M) with numerous projections are accumulated within the alveolar lumen (AL). Some of them are migrating through the indistinct borders of the alveolar walls into the lumen. Open stars show probable borderlines of the alveolar walls. ×2,185.

Fig. 10. Rat paraquat lung (10 days after injection). Fibroblasts (F) or myofibroblasts (Mf) proliferate within the alveolar lumen. R: repaired epithelial cell. ×2,660.
are rather dilated, because intraductal fibrosis hardly takes place.

Ultrastructurally, minor but definite degenerative change occurs in the epithelial cells of alveoli before the histologic changes become detectable. The initial change is hydropic swelling of the type 1 pneumocytes, which is observed already 6 hours after the administration of paraquat (Fig. 3). The hydropic epithelial cells gradually increase in number and intra-alveolar edema becomes apparent. On the 3rd day the necrotic epithelial cells are desquamated over wide areas. In most parts of the denuded alveolar surface the epithelial basal lamina remains intact (Figs. 4, 5), but there is often adherent hyaline membrane (Fig. 6), which is a densely accumulating cell debris occasionally associated with fibrin deposits on the alveolar surface (Fig. 7). On the 5th day macrophages migrating into the alveolar spaces often engulf the cell debris. The remaining type 2 pneumocytes begin to proliferate and spread along the denuded surface (Fig. 8). On the other hand, severe damage in the epithelial layers makes the air-tissue border unclear according as the basal lamina disappears, and there are many macrophages infiltrating into the alveolar spaces (Fig. 9). The macrophages have abundant rough ER in their cytoplasm, which is suggestive of their activation. After the 7th day fibroblasts next migrate into the alveolar lumen and gradually increase in number. On the 10th day the original alveolar spaces are replaced with proliferating fibroblasts associated with a fair amount of pericellular collagen fibers (Fig. 10). The fibroblasts contain abundant rough ER which is occasionally filled with amorphous material. Some of these cells contain myofilament bundles in the cytoplasm, and are therefore regarded as myofibroblasts (Fig. 11).

The sequential changes occurring in the alveoli after initial epithelial damage are schematically shown in Fig. 12. The damaged epithelial cells form characteristic hyaline membranes resulting from condensation of their debris. The necrotic masses probably induce marked extravasation of blood monocytes and intra-alveolar accumulation of macrophages. Activated alveolar macrophages could conceivably contribute to the recruitment and proliferation of the septal cells which are preferentially active in collagen production. The remodeling of alveolar structure due to
intra-alveolar fibrosis finally forms a new broad septum resulting from the cicatricial collapse of the organized alveolar spaces. The cicatricial collapse of the original alveolar spaces is partially due to the contractile ability of the increased myofibroblasts. No morphologic evidence of endothelial damage is found in any of the experiments with paraquat except for increased vascular permeability.

**Endothelial damage to the alveoli**

The alveolar capillaries may be very susceptible to many toxic agents which are transported through the circulation into the lungs. Monocrotaline\(^\text{44-41}\), alpha-naphtyl thiourea\(^\text{42,43}\), and butylated hydroxytoluene\(^\text{44,45}\) are perhaps the most thoroughly studied of the putative endothelial toxins. Monocrotaline is one of the pyrrolizidine alkaloids which is metabolized in the liver and becomes a highly reactive metabolite, monocrotaline pyrrole. It is well known that this metabolite on reaching the lungs injures mainly the endothelial cells of the microcirculation by its alkylation effects.

A single subcutaneous injection of a high dose (from 40 mg/kg to 120 mg/kg) of monocrotaline in rats can produce DAD in slow sequence. Severe lung edema begins 3 days after the injection and continues for 2 weeks. Necrotizing inflammation of the alveolar septum associated with fibrin deposits and hyaline membranes occurs in places after the 3rd week of the experiment (Fig. 13a). The rats surviving for almost one month develop interstitial fibrosis and occasional epithelial hyperplasia (Fig. 13b). The main cause of death in the monocrotaline-treated rats is cor pulmonale which is induced by pulmonary hypertension, because monocrotaline pyrrole also severely affects the walls of small pulmonary arteries.

The initial changes which are electron-microscopically recognized 4 to 6 hours after the injection are blebs and gaps in the endothelial cells. These changes are observed in the pulmonary microvessels, mainly in alveolar capillaries and venules but to a lesser degree in arterioles. Many capillaries are nearly occluded by enlarged blebs protruding into the capillary lumen (Fig. 14). There is severe subendothelial edema, in which endothelial cells are separated from the basal lamina and exudative fluid is accumulated. On the 3rd day hydropic swelling of the endothelial cells, with marked nuclear and cytoplasmic changes, is added to the bleb formation (Figs. 15, 16). Occasionally, necrotic endothelial cells and denuded spaces are seen along the microvascular lumen, where platelet and/or fibrin thrombi are present. Interstitial edema of the alveolar walls gradually increases as the endothelial changes extend. Although the capillary degeneration is prominent, the other cells of the alveolar walls are
Fig. 13. Light micrographs of rat lung injured by monocrotaline, showing necrosis of the alveolar walls; (a) exudative stage, and (b) proliferative stage showing mainly interstitial fibrosis. H.E., ×216.

Fig. 14. Rat monocrotaline lung (6 hours after s.c. injection). A large bleb (B) of capillary endothelium, the initial change in rat lungs treated with monocrotaline, is protruding into a capillary lumen (CL). AL: alveolar lumen. ×12,350.
Fig. 15. Rat monocrotaline lung (14 days after injection). The capillary endothelial cells (En) decrease in density and are swollen, but the alveolar epithelial cells (Ep) appear normal. AL: alveolar lumen, C: capillary. ×13,300.

Fig. 16. Rat monocrotaline lung (14 days after injection). This shows hydropic degeneration of the endothelial cells (En) of a pulmonary venule. V: venule, Ly: lymph vessel, AL: alveolar lumen. ×3,420.
Fig. 17. Rat monocrotaline lung (21 days after injection). Near a severely damaged capillary epithelial cells (Ep) and septal cells (S) are swollen and there are numerous lipid droplets within the cytoplasm. The capillary is filled with platelet (P) and fibrin (F) microthrombi. ×2,850.

Fig. 18. Rat monocrotaline lung (21 days after injection). This shows hydropic and fatty degeneration of endothelial cells (Ep) associated with destruction of the alveolar capillaries. Arrowheads: fat droplets, FT: fibrin thrombus, M: macrophage. ×3,800.
Fig. 19. Schematic presentation of sequential alveolar changes following the initial endothelial damage. Phases 1 & 2, bleb formation and degeneration of the capillary endothelium with interstitial edema; phase 3, epithelial damage and formation of hyaline membrane with marked fibrin deposits; phase 4, macrophage reaction in the destroyed septum; phases 5 & 6, interstitial fibrosis resulting from fibroblast proliferation.

almost normal for two weeks after the injection. These results may indicate that the changes seen after 3 weeks are a secondary reaction.

After the 3rd week of the experiment the cell damage significantly extends to epithelial and septal cells near the damaged capillaries (Fig. 17). The damaged epithelial and septal cells become severely hydropic and many fat droplets appear within the cytoplasm (Fig. 18). In the severely damaged foci most cellular components are involved in necrosis and there are fibrin deposits in the interstitial spaces as well as in the capillary lumen. These lesions are histologically seen as necrosis of the whole septal tissue. As the epithelial damage progresses, fibrin-rich hyaline membranes are attached to the necrotic alveolar septum.

The sequence of alveolar changes following the initial monocrotaline-induced endothelial damage is schematically shown in Fig. 19. Phases 1 and 2 show the endothelial damage including bleb formation, hydropic changes of endothelial cells, occasional formation of capillary thrombi, and interstitial edema. Phases 3-6 show the sequential changes of the alveolar septum following secondary epithelial damage. In the lung injury initiated by endothelial damage, intra-alveolar fibrosis is less marked than in that due to primary epithelial damage, because the secondary epithelial destruction is limited to areas in which the capillaries are severely destroyed. No remodeling of the alveolar structure occurs except in the lesions where the alveolar surface is destroyed extensively. The final feature of the endothelial damage is usually “interstitial” fibrosis.

Severe alveolar damage involving the whole alveolar wall

Some toxic gases such as oxygen, ozone, and alloxan have been reported to affect both epithelial and endothelial cells simultaneously. The general histologic features of alveolar damage by these agents are apparently similar to those described in the section on epithelial damage. However, for many agents the primary site of injury and the factors contributing to the subsequent changes have not been well characterized.

Mechanisms of chemically induced lung injury involving metabolic activation

Boyd has reported three potential mechanisms of lung toxicity in which metabolic activation plays a crucial role. Mechanism I depicts a reaction in which an “inert” parent compound is metabolized to a highly reactive ultimate toxin in situ in the lung. In Mechanism II, the ultimate toxin is also a highly reactive metabolite of the parent compound, but is formed primarily in the liver and is transported to the lung by the circulation. In Mechanism III, the parent compound
undergoes a cyclic reduction/oxidation leading to the "activation" of molecular oxygen and the consumption of cellular-reducing equivalents in the alveolar cells. Thus, in the third mechanism, the parent compound participates only indirectly in the toxic reaction. Presumably, the activated species of oxygen could directly act as the ultimate toxin in the lung. The lung injuries caused by monocrotaline and paraquat which are demonstrated in this paper are the best available examples of toxicities in accordance with Boyd's Mechanism II and III, respectively.

Proposed mechanism of secondary epithelial damage following endothelial damage

As described in the monocrotaline experiment there are long intervals between the initial appearance of endothelial damage and the occurrence of epithelial damage. The latter change is too late to be explained as the result of the direct injury by monocrotaline-metabolite. In other words, both types of cell would be damaged simultaneously, if they were affected soon after the injection of monocrotaline. Most monocrotaline and its metabolites are excreted in the urine for 24 hours after administration. Therefore, the delayed epithelial damage has been considered to be secondary. However, the mechanism of the secondary epithelial damage has not been clarified.

Here we will discuss the nature of the secondary reaction from the standpoint of breaking down the antioxidation system during the stage of pulmonary endothelial damage. The lung is an organ which is highly exposed to oxygen. The anti-oxidation system of the tissue is important especially in the lung to prevent preferential damage by activated species of oxygen (superoxide and its derivatives). A diminished defense mechanism could lead to lung injury. The lung tissue of normal animals contain a fair amount of various antioxidation enzymes for activated oxygen, such as superoxide dismutase, catalase, and glutathione peroxidase.

Many experimental studies on hyperoxia have clarified the mechanism of the lung injury induced by oxygen-related free radicals. The potential toxicity of the superoxide anion has been attributed to its free radical status. Reactions involving the superoxide anion radical may produce other potentially toxic species such as the hydroperoxy radical, hydroxyl radical, hydrogen peroxide, and singlet oxygen. In addition, hydrogen peroxide is produced through the dismutation of superoxide anion. It is believed that interaction of superoxide anion and/or other reduction products of oxygen with unsaturated tissue lipids, which are an important component of cell membranes, may result in the extraction of an electron from the lipid, producing a lipid free radical. The net result of this cyclical series of reactions is the production of a lipid peroxide and lipid free radical, resulting in progressive cell damage. Figure 20 shows a simplified schema of the production of lipid peroxide in the tissue.

![Fig. 20. Representation of metabolic events accompanying exposure to excessive oxygen-related free radicals.](image-url)
Figure 21. (a) Activities (units/mg protein) of SOD and catalase in the lungs of rats treated with a single s.c. injection of monocrotaline (50 mg/kg). (b) Contents of lipid peroxide (mol/g tissue), contents of glutathione (µmol/g tissue), and activities of glutathione peroxide (units/mg protein) in the rat lungs demonstrated in (a). Each SD is calculated from data of five animals 1 week to 5 weeks after the injection.

Figure 21a shows the activities of superoxide dismutase and catalase in the rat lung following a single injection of monocrotaline. The activities of both antioxidation enzymes show clear depletion from the early days, which coincides with the stages of endothelial damage. Figure 21b shows the amounts of lipid peroxide and glutathione and the activity of glutathione peroxidase with the passage of time. The amounts of lipid peroxide and glutathione are not depleted until the 4th week of the experiment. The activity of glutathione peroxidase, a scavenger of glutathione, is coincidentally involved in induction. It is interesting that the latter findings concerning lipid peroxidation occur in the stage of secondary epithelial damage. There is a possibility that the progressive endothelial damage may finally induce the failure of the antioxidation system of the lung, which may lead to progression of tissue damage. Increased lipid droplets in the damaged epithelial cells may be evidence of lipid peroxidation within the cells, although lipid peroxidate cannot be morphologically demonstrated in the specimens.

Pathogenesis of lung fibrosis following alveolar damage

The connective tissue cells in the alveolar walls are called septal cells, fibroblasts, or often myofibroblasts. These cells have the power of collagen production, although their cellular features in normal lungs are a little different from those of fibroblasts which are seen in the perivascular or peribronchial areas of the lung. Unlike the type I pneumocytes and endothelial cells, the septal cells are relatively resistant to injury. Therefore, the proliferating fibroblasts in the alveolar regions following alveolar damage may originate from these cells. The fact that fibroblasts seen in the proliferative stage of DAD usually contain neither lipid droplets nor myofilaments in their cytoplasm probably indicates that the septal cells can change their nature under altered conditions. In cultured septal cells taken from normal peripheral lungs, cytoplasmic lipid droplets are hardly found in the conventional medium, but significantly increase in a vitamin A-added medium.

Much in vitro evidence has accumulated that a variety of chemical mediators are concerned with proliferation of fibroblasts. The exudate which increases in the exudative stage of DAD may contain cell growth factors such as PDGF. In fact, it is observed in animal and human lungs that delayed interstitial edema produces interstitial
fibrosis in the alveolar regions.

Recent studies have indicated that the macrophage probably plays a major role in the process of post-inflammatory fibrosis. This hypothesis may be adopted in the case of lung fibrosis following DAD\textsuperscript{18,19}. As demonstrated in lungs affected by paraquat, many macrophages infiltrate into the alveolar spaces of injured sites before the proliferation of fibroblasts. It can be shown ultrastructurally that macrophages can recruit fibroblasts into the alveolar spaces and stimulate the newly recruited cells. Recent studies of idiopathic interstitial pneumonia of man have reported that alveolar macrophages are capable of releasing two mediators, fibronectin\textsuperscript{54} and the alveolar macrophage-derived growth factor (AMDGF)\textsuperscript{55}, which together can modulate the expansion of fibroblast numbers in the injured alveolar sites\textsuperscript{56}.

We examined in our \textit{in vitro} study the influence of macrophages on the alveolar septal cells which were established as a cell line in our laboratory. The conditioned medium of macrophages taken from paraquat-injured rat lungs accelerates proliferation of the alveolar septal cells. This finding suggests that macrophages activated in alveolar damage may secrete growth factor or factors influencing the proliferation of the septal cells, which are considered to be the primary connective cells in alveolar fibrosis.

The nature of epithelial and endothelial damage in the lung

It has been demonstrated in the two experimental models in this article that the alveolar damage may take different courses on the basis of the target cells of toxic agents, either epithelial or endothelial cells. While the lung injury affecting the epithelial cells may produce a typical feature of DAD from the early stage, in the injury initiated by endothelial damage the air-blood barrier remains unaffected until the epithelial cells are secondarily damaged, and so DAD may be delayed.

The agents which are involved in production of oxygen free radicals in the lung like paraquat cause severe epithelial damage from the early stage. The epithelial damage causes destruction of the air-blood barrier, resulting in rapid development of a respiratory failure. Histologically, it shows a typical feature of DAD with dominant hyaline membrane formation. The alveolar damage of this type shows a similar feature to that in hyperoxia. The animals surviving more than one week are involved in intra-alveolar fibrosis and remodeling of alveolar structure, because of severe destruction of the alveolar walls.

On the other hand, many other exogenous chemical compounds given through roots other than airway may be metabolized in the liver. Some of them are altered to active metabolites, which are transferred into the pulmonary circulation and first come into contact with the endothelial cells of the pulmonary vessels. Thus, it is easily understood that these active metabolites may affect the endothelial cells more severely than the epithelial cells in the lung. It is well known in human beings that most drug-induced lung injuries have a clinically silent period before physiological abnormalities occur, through the period is variable by the nature and dose of drugs. The reason why the lung injury is delayed is not fully understood. However, our observations on monocrotaline-induced lung injury suggest that endothelial damage may induce secondary epithelial damage after some duration. The secondary epithelial damage may need the other additional condition for cell damage, as breaking down of antioxidation system seen in the monocrotaline-induced lung injury. Once the alveolar epithelium is damaged, the air-blood barrier is destroyed and histologically DAD develops.

Whether it is a primary or a secondary effect of the toxic agents, the epithelial damage is a common change of the lung injuries, histologically regarded as DAD. Hyaline membrane, which is fairly characteristic of DAD, is formed more or less at any time that epithelial damage occurs, because this membrane is an accumulation of cell debris which may be mechanically condensed on the alveolar surface. The pneumotoxins of endothelial type produce DAD after epithelial damage secondarily develops.

The fibrosis following DAD has usually been described as an interstitial fibrosis. Although this type of fibrosis is one of the sequential changes of DAD, intra-alveolar fibrosis is more common in
pulmonary fibrosis is a combined interstitial and
with no or mild epithelial damage. Most human
alveolar walls may be present only in the cases
our studies that pure interstitial fibrosis of the
final stage. It is considered on the basis of
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